

The Course of Biodegradation of Anionic Detergents by Analyses for Carbon, Methylene Blue Active Substance and Sulfate Ion¹

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T. C. CORDON, E. W. MAURER and A. J. STIRTON,
Eastern Utilization Research and Development Division,² Philadelphia, Pennsylvania 19118

Abstract

Tallow alcohol sulfates, ether alcohol sulfates and esters of α -sulfo tallow fatty acids were degraded aerobically by sewage microorganisms in a system in which detergent was the sole source of C. Biodegradation was followed by loss of C and methylene blue active substance (MBAS) and formation of SO_4^{--} . Tallow alcohol sulfates were rapidly and completely degraded; ether alcohol sulfates not quite as readily. Reduction in MBAS was rapid for the α -sulfo esters but loss of C and SO_4^{--} formation was incomplete, possibly because of the intermediate formation of a resistant sulfosuccinate. Sodium *p*-(1-methylundecyl)benzenesulfonate (LAS) was the reference standard.

Introduction

Studies by this laboratory have been reported on the biodegradation of tallow-based detergents in river water (7), in a laboratory scale activated sludge system (3), and in anaerobic digesters (5). An investigation of the metabolism of tallow-based detergents by sewage microorganisms (1) has now been extended to give some information on the mechanism of their breakdown under aerobic conditions. The tallow-based detergents were tallow alcohol sulfates, ether alcohol sulfates and esters of α -sulfo fatty acids. Other compounds were included to help relate structure with ease of biodegradation. The individual isomer sodium *p*-(1-methylundecyl)benzenesulfonate (LAS) was a reference standard.

The Esso Research test (4), with the detergent as the sole source of C, was the basis for a method which permitted measurement of inherent turbidity and analyses for C, methylene blue active substance (MBAS), and SO_4^{--} during the course of biodegradation.

Experimental Procedures

The tests were carried out in widemouth jars containing 3 liters of deionized water, 30 mg of inoculum, nutrient salts free of sulfate and 120 mg of detergent.

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² ARS, USDA.

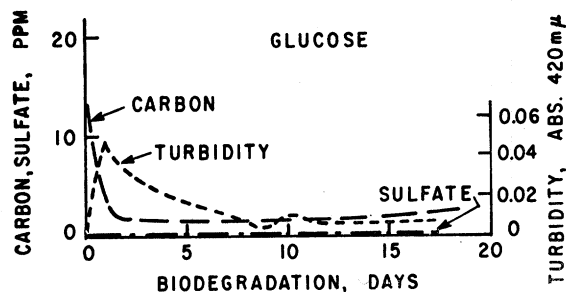


FIG. 1. Biodegradation of glucose.

The contents were stirred continuously by magnetic stirrers to insure adequate aeration. Oxygen content, checked with an oxygen meter, was near the saturation point for the test temperature. The activated sludge inoculum was prepared by running sewage samples for one week by a procedure used in confirming tests (6).

Carbon

Total C was determined with a Beckman Carbonaceous Analyzer. Samples were prepared for analysis by centrifuging at 4500 rpm to remove particulate matter, including cells of microorganisms, and the supernatant was purged with N_2 to remove CO_2 . In cases where the detergent was not completely soluble the sample was heated to about 80°C to dissolve the detergent, and centrifuged warm.

MBAS

A Technicon Automatic Analyzer was used. Transmittance was compared with reference standards and expressed as ppm detergent remaining. Samples were used as taken from the aerators without treatment.

Sulfate Ion

Sulfate ion was determined by a turbidimetric method developed in this laboratory (2). The use of isopropyl alcohol instead of absolute ethyl alcohol has been found recently to give a more nearly linear standard curve.

Turbidity

In the method for SO_4^{--} it was necessary first to measure turbidity in the presence of all reagents except BaCl_2 . The values were subtracted from total turbidity before SO_4^{--} values were computed. The inherent turbidity may give information about the breakdown of detergents and these absorbance values

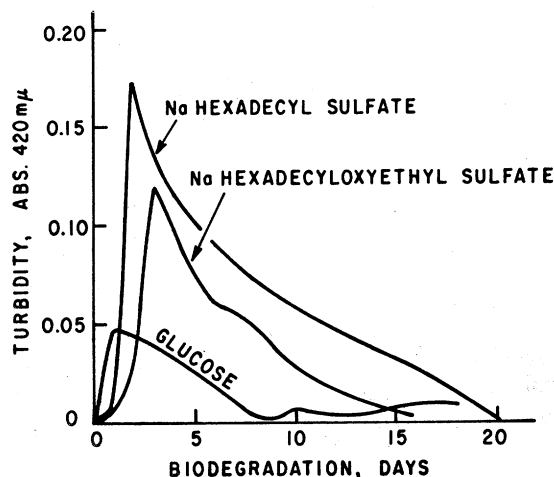


FIG. 2. Comparative turbidities during biodegradation.

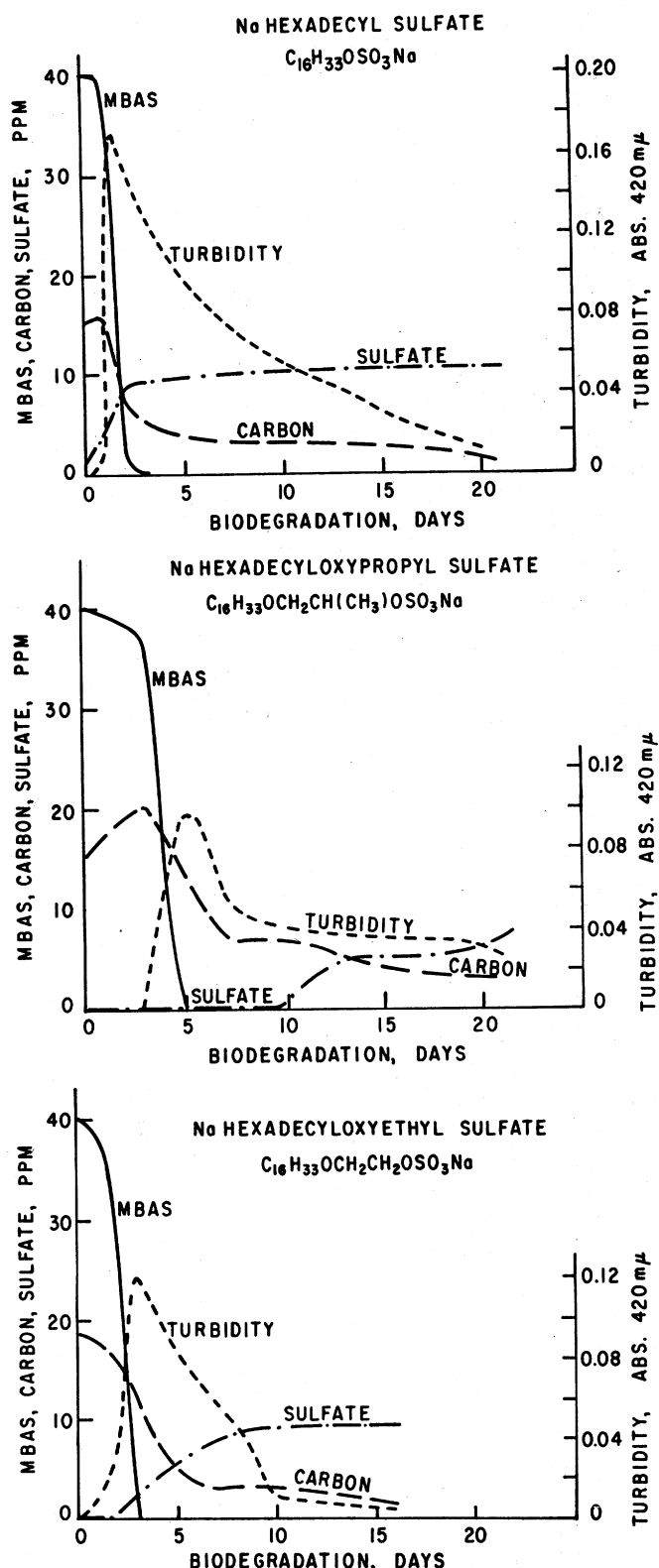


Fig. 3. Biodegradation of alcohol sulfates and ether alcohol sulfates.

were therefore recorded. Hereafter reference to turbidity in this paper is to inherent turbidity.

Results and Discussion

Turbidity may be caused both by the growth of microbial cells and by the formation of insoluble compounds from detergent degradation. In Figure 1 the growth of microbial cells in the biodegradation of

TABLE I
Carbon Loss, MBAS Reduction and Sulfate Ion Formed in the Biodegradation of Anionic Detergents

No.	Detergent	Carbon lost, % of theory	SO ₄ ²⁻ formed, % of theory	MBAS* Days required for reduction to zero ppm
1	Na hexadecyl sulfate $C_{16}H_{33}OSO_3Na$	94	96	2
2	Na hexadecyloxyethyl sulfate $C_{16}H_{33}OCH_2CH_2OSO_3Na$	95	98	3
3	Na hexadecyloxypropyl sulfate $C_{16}H_{33}OCH_2CH(CH_3)OSO_3Na$	82	73	5
4	Na hexadecyloxybutyl sulfate $C_{16}H_{33}OCH_2CH_2CH_2CH_2OSO_3Na$	91	94	5
5	LAS ^b (Ave. of 9 runs) (range)	89 86-95	84 71-92	9
6	Na dodecanesulfonate $C_{12}H_{25}SO_3Na$	96	100	4
7	Na N-methyl-N-sulfoethylpalmitamide $C_{15}H_{31}CON(CH_3)C_2H_4SO_3Na$	94	94	4
8	Na ₂ sulfoacetate $NaO_2SCH_2CO_2Na$	100	100	*
9	Na dodecyl sulfoacetate $C_{12}H_{25}O_2CCH_2SO_3Na$	97	96	3
10	K hexadecyl α-sulfoacetate $C_{16}H_{33}O_2CCH(SO_3Na)CH_3$	92	91	3
11	Na tetradecyl α-sulfoacetate $C_{14}H_{29}O_2CCH(SO_3Na)CH_3$	58	0	5
12	Na hexyl α-sulfoacetate $C_7H_{15}CH(SO_3Na)CO_2C_6H_{13}$	80	22	9
13	Na dodecafluorooctyl α-sulfoacetate $C_{12}H_{15}CH(SO_3Na)CO_2CH_2(CF_2)_6CHF_3$	48	3	
14	Na ₂ α-sulfostearate $C_{16}H_{33}CH(SO_3Na)CO_2Na$	70	0	^d
15	Na methyl α-sulfostearate $C_{16}H_{33}CH(SO_3Na)CO_2CH_3$	87	48	5
16	Na isopropyl α-sulfostearate $C_{16}H_{33}CH(SO_3Na)CO_2CH(CH_3)_2$	74	23	7
17	Na ₂ 2-sulfoethyl α-sulfostearate $C_{16}H_{33}CH(SO_3Na)CO_2C_2H_4SO_3Na$	68	20	7
18	Na sulfosuccinic acid $HO_2CCH_2CH(SO_3Na)CO_2H$	36	0	^e
19	Na dioctyl sulfosuccinate $C_8H_{17}O_2CCH_2CH(SO_3Na)CO_2C_8H_{17}$ ^a	83	0	10

^a Methylene blue active substance.

^b Na p-(1-methylundecyl)benzenesulfonate.

^c Not surface active. No color reaction with methylene blue.

^d Not determined.

^e Aerosol OT.

glucose reached a maximum value of 0.05 on the absorbance scale in one day and then decreased. On the 16th day a measurable amount of "sulfate" was present. False sulfate values of up to 2 ppm which sometimes appear and later disappear during detergent biodegradation may be due to microbial growth as in the case of glucose.

Alcohol sulfates and ether alcohol sulfates, compared with glucose in Figure 2, showed much larger maximum turbidity values possibly due to the separation of insoluble long chain alcohols and ether alcohols.

Alcohol Sulfates and Ether Alcohol Sulfates

Sodium hexadecyl sulfate was rapidly attacked as shown in Figure 3. The MBAS dropped to almost zero in two days and turbidity reached a maximum of 0.175 on the absorbance scale at the same time. Soluble carbon decreased to 27% of the theoretical value and 90% of the theoretical SO₄²⁻ was formed. From this point on the reaction slowly approached completion so that after 20 days turbidity was only 0.01 on the absorbance scale, 94% of C had been lost and 96% of theoretical SO₄²⁻ was present.

The curves for the ether alcohol sulfates were similar to that for the alcohol sulfate. Biodegradation was not rapid but proceeded essentially to completion, except that for sodium hexadecyloxypropyl sulfate about 25% of theoretical SO₄²⁻ did not appear and 20% of theoretical C remained. There was no significant difference between sodium hexadecyloxyethyl sulfate and sodium octadecyloxyethyl sulfate.

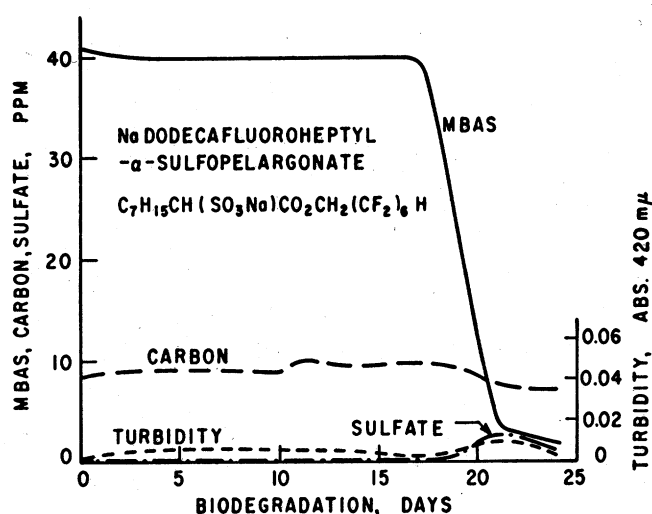
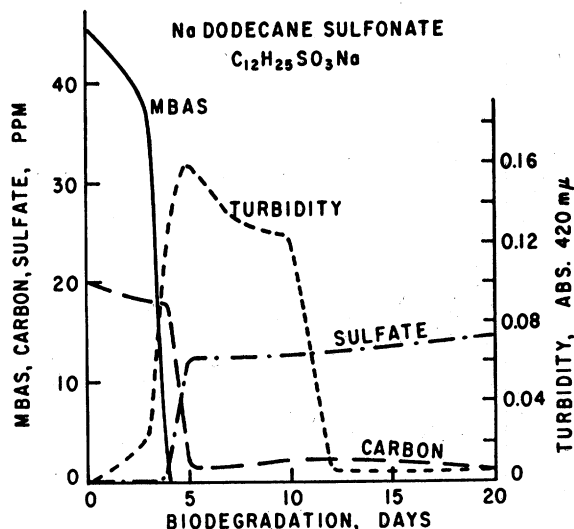
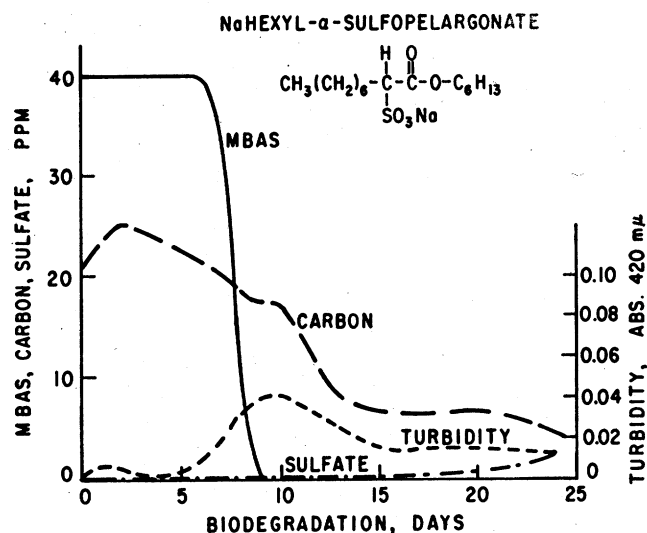
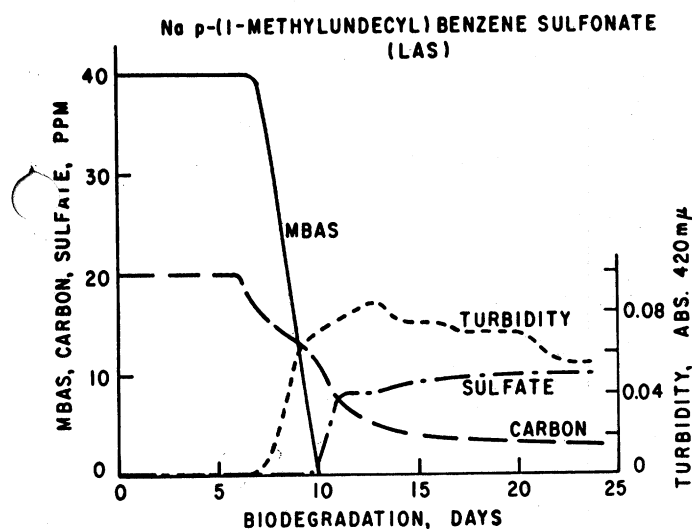


FIG. 4. Biodegradation of LAS, Na dodecanesulfonate and Na dodecyl sulfoacetates.

Aliphatic and Aromatic Sulfonates

As shown by Figure 4, biodegradation of sodium dodecanesulfonate was more rapid and complete than for a linear alkylbenzenesulfonate. The value for MBAS dropped to zero in three to four days compared to nine days for LAS; and loss of C and SO_4^{--} formation was nearly quantitative compared to 89% and 84% respectively for LAS. This is true also of other surface active aliphatic sulfonates, not graphed but listed in Table I; specifically sodium N-methyl-N-(2-sulfoethyl)palmitamide (No. 7), sodium dodecyl sulfoacetate (No. 9), and potassium hexadecyl α -sulfopropionate (No. 10).

Esters of α -Sulfo Fatty Acids

With the exception of the fluoro compound (No. 13) all of the α -sulfo esters shown in Figures 5, 6, 7 and Table I, including the sulfosuccinates, were aerobically biodegradable in the sense that MBAS was reduced to zero. Biodegradation was complete for the α -sulfo stearates and α -sulfo propionates which showed nearly quantitative loss of C and formation of SO_4^{--} . The α -sulfo butyrates and higher homologs of the α -sulfo monocarboxylic acids were less completely degraded, with loss of about 60% to 90% of the theoretical C and formation of 20% to 50% of theoretical SO_4^{--} . This may be due to the intermediate

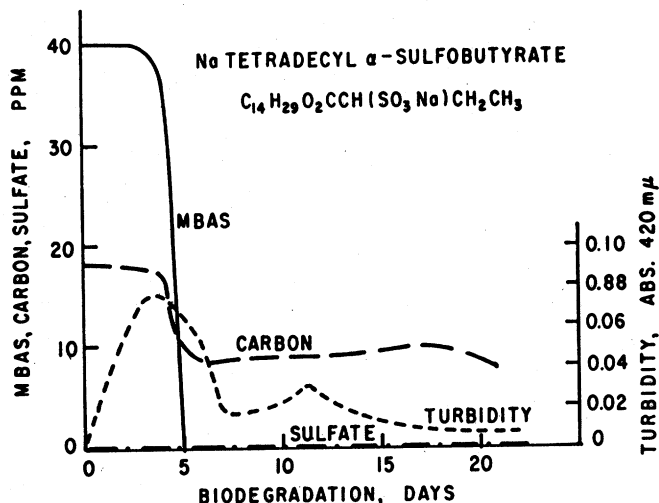


FIG. 5. Biodegradation of α -sulfo butyrates and α -sulfo pelargonates.

formation of sulfosuccinates which although biodegradable to the point of loss of surface active properties were resistant to any further decomposition. The sulfo group in this case apparently remains intact without formation of SO_4^{--} . According to this concept, for which there is not much evidence at present, aerobic biodegradation of sulfoacetate, α -sulfo propionates and α -sulfo butyrates may proceed as follows:

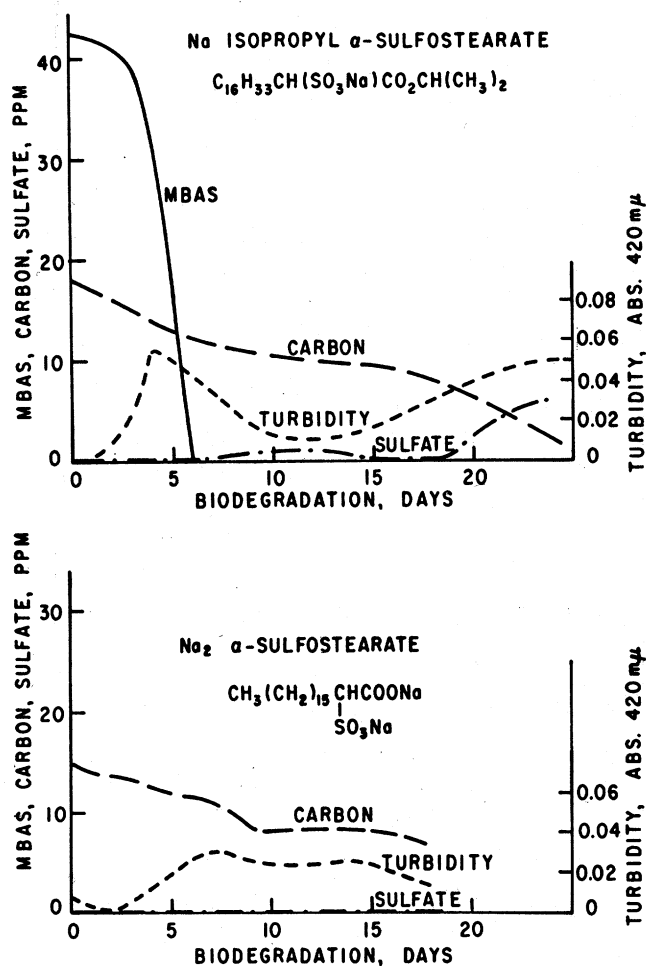


FIG. 6. Biodegradation of α -sulfostearates.

1. $RO_2CCH_2SO_3Na \longrightarrow HO_2CCH_2SO_3Na \longrightarrow CO_2 + H_2O + SO_4^{--} + Na^+$
2. $RO_2CCH(SO_3Na)CH_3 \longrightarrow HO_2CCH(SO_3Na)CO_2H \longrightarrow HO_2CCH_2SO_3Na \longrightarrow CO_2 + H_2O + SO_4^{--} + Na^+$
3. $RO_2CCH(SO_3Na)CH_2CH_3 \longrightarrow HO_2CCH(SO_3Na)CH_2CO_2H$

α -Sulfopelargonates, laurates, myristates, palmitates and stearates may degrade through hydrolysis and oxidation to sulfosuccinates which then remain quite resistant to further reaction. Sulfosuccinates are generally considered nontoxic and are useful in the food industry.

α -Sulfo esters such as sodium hexyl α -sulfopelargonate which are wetting agents with the hydrophilic group near the middle of the molecule have

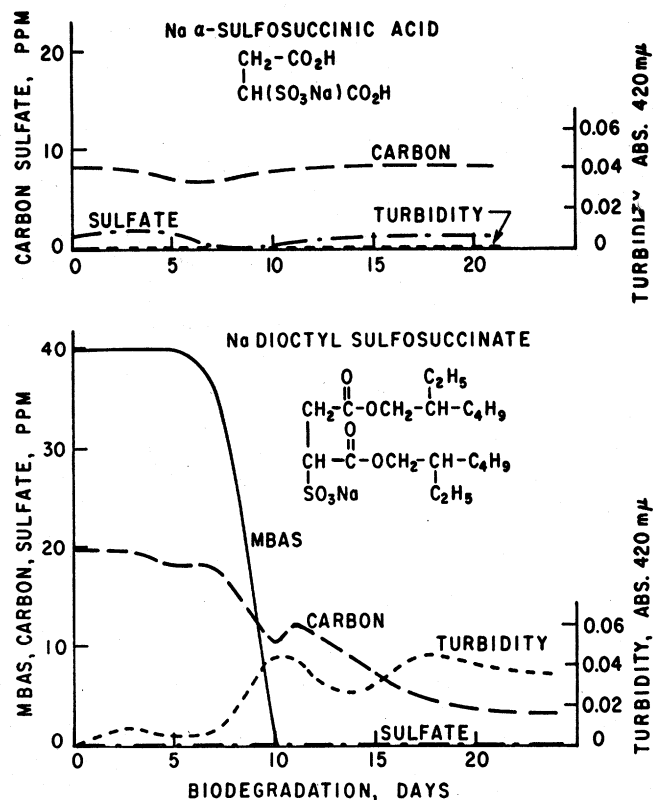


FIG. 7. Biodegradation of sulfosuccinates.

been shown to be less readily biodegradable than α -sulfo esters of the detergent type (7). This is shown again in Table I and in a comparison of Figures 5 and 6. Sodium dodecafluoroheptyl α -sulfopelargonate, also an effective wetting agent may be toxic to microorganisms. Turbidity, as a sign of microbial cell growth remained at a very low level and as a consequence almost no biodegradation occurred until after a period of about 18 days.

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